Based on the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Information Disclosure Statement

Applicants note that the Examiner did not initial all of the documents cited in the First Supplemental Information Disclosure Statement filed on August 28, 2000. (*See* copy of Information Disclosure Statement attached to Paper No. 12.) Applicants respectfully request that the Examiner initial the documents cited in the Information Disclosure Statement or indicate why they were not considered.

Rejections under 35 U.S.C. § 112

The Examiner rejected claims 1, 3-10, 13, 16-17, 19, 26, 28-29 and 34-44 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. (Paper No. 12, at page 2.) The Examiner alleged that

> [c]laims 1, 3-10, 13, 16, 17, 19, 26, 28, 29 and 34-37 are directed to all possible *Thermotoga maritima* (*Tma*) DNA polymerase mutants which are modified at least two ways selected from the group consisting of (a) to reduce or eliminate the 3'→5' exonuclease acitivity of the polymerase; (b) to reduce or eliminate the 5'→3' exonuclease activity of the polymerase; and (c) to reduce or eliminate discriminatory behavior against a dideoxynucleotides (claims 1 and 3-10), and methods of using and kits comprising said DNA polymerase mutants (claims 17, 19, 26, 28, 29, 34, 35 and 36) and genes encoding said DNA polymerase mutants (claims 13, 16 and 37). . . . There is no disclosure of any particular structure to function/activity relationship in the claimed genus. The specification also fails to describe additional representative species of these DNA Polymerases

> by any identifying structural characteristics or properties other than having reduced 3'→5' exonuclease activity of the polymerase, reduced 5'→3' exonuclease activity or reduced discriminatory behavior against dideoxynucleotides for which no predictability of structure is apparent. Given this lack of additional representative species as encompassed by the claims, Applicants have failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention.

Id. at pages 2-3. The Examiner similarly rejected claims 38-44. (*See id.* at pages 3-4.)

Applicants respectfully disagree with the Examiner and submit that one skilled in the art could reasonably conclude that Applicants had possession of the *Tma* DNA polymerase mutants encompassed by the rejected claims, in the present application as filed.

The test for the written description requirement is whether one skilled in the art could reasonably conclude that the inventor has possession of the claimed invention in the specification as filed. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 U.S.P.Q.2d 1111, 1116 (Fed. Cir. 1991); M.P.E.P. § 2163.02. The Examiner bears the initial burden of presenting a *prima facie* case of unpatentability. This burden is discharged if the Examiner can present evidence or reasons why one skilled in the art would not reasonably conclude that Applicants possessed the subject matter as of the priority date of the present application. *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ2d 90, 96 (C.C.P.A. 1976); M.P.E.P. § 2163.04. In the instant case, the Examiner has not met this burden.

Applicants submit that the Examiner has underestimated both the teaching of the present application and the level of skill in the art on the priority date of the present application. Importantly, the Examiner has done nothing more than to argue lack of literal support in the application and has failed to meet his burden in presenting evidence or reasons why one skilled in the art would not reasonably conclude that Applicants were in

possession of the subject matter of the rejected claims on the priority date of the present application.

Applicants submit that the standard for fulfilling the written description requirement is whether the specification provides sufficient disclosure for one skilled in the art to readily envision a representative number of members of the claimed genus. *See* Revised Interim Utility Guidelines Training Materials, at page 9. Here, the Examiner has not met the burden of showing that one of skill in the art could not envision a representative number of species of the claimed genus.

Applicants submit that at the time the invention was made, the sequence of many DNA polymerases had been compiled and aligned. These DNA polymerases can be divided into several distinct families based on sequence homology. For example, the *Tne* and *Tma* DNA polymerases are part of the Pol I-type family of polymerases and exhibit significant homology to *E. coli* DNA polymerase I.

Pol I-type DNA polymerases share many conserved sites throughout the protein. Gutman *et al.*, *Nucl. Acids Res. 21*:4406-4407 (1993) (reference AT3) describe conserved sites in the 5'-3' exonuclease domain of Pol I-type DNA polymerases. Six conserved regions, identified as regions A-F, are shown in Figure 1 of Gutman *et al.* and a consensus sequence for each of these conserved regions is provided. A review of the *Tma* DNA polymerase amino acid sequence, as provided in U.S. Patent No. 5,374,553 (which is incorporated by reference as indicated at page 18 of the specification), shows significant homology to these consensus sequences.

Similarly, Bernad *et al.*, *Cell 59*:219-228 (1989) (reference AS1), describes conserved regions in the 3'-5' exonuclease domain of Pol I-type polymerases. Significant similarity between these conserved regions and corresponding regions in the *Tma* DNA

polymerase can be seen, e.g. in the Exo I and Exo II regions. Each of these regions exhibits a high degree of homology to the Exo I and Exo II regions of other Pol I-type polymerases, as described in Bernad.

All of the above would provide information to a scientist skilled in the art regarding the domain structure of the *Tma* DNA polymerase, including the localization of the exonuclease activities and polymerase activity to specific domains. For example, the 5'-3' exonuclease domain of *E. coli* polymerase I is at the N-terminal end of the polymerase, while the middle and C-terminal domains of this polymerase exhibit 3'-5' exonuclease and DNA polymerase activity, respectively. *See,* C. M. Joyce, *Curr. Opin. Str. Biol. 1*:123-129 (1991) (reference AR5). This domain structure is conserved in other Pol I-type polymerases, such as *Tma* DNA polymerase.

Further information regarding the domain structure of the *Tma* DNA polymerase can be seen from the functional characterization of deletion mutants of *Tne* DNA polymerase and several other Pol I-type polymerases. For example, deletion of the first 235 and 288 amino acids from the N-terminal region of *Taq* polymerase eliminates 5'-3' exonuclease activity. Similarly, deletion of the first 250 amino acids of the *Tth* polymerase and the first 323 amino acids of *E. coli* polymerase I also eliminates 5'-3' exonuclease activity. Deletion of the first 514 and 520 amino acids from the N-terminal region of *E. coli* polymerase I eliminates both 5'-3' and 3'-5' exonuclease activity.

Thus, the sequence and other information disclosed for DNA polymerases in the specification would indicate to a scientist skilled in the art that polymerase activity is localized to the C-terminal portion of the *Tma* DNA polymerase, while the 5'-3' and 3'-5' exonuclease activities are localized to the N-terminal and middle portions of this polymerase, respectively. Fragments containing the C-terminal region will typically retain

polymerase activity, while fragments lacking the 5'-3' or 3'-5' exonuclease domains will not retain 5'-3' or 3'-5' exonuclease activity, respectively.

In addition, significant additional information regarding mutant forms of *Tma* DNA polymerase with reduced or no 5'-3' or 3'-5' exonuclease activity, or reduced or no discriminatory behavior against a dideoxynucleotide can be obtained when coupled with structural information that has been obtained for *E. coli* polymerase I and other Pol I-type DNA polymerases. For example, a high resolution three-dimensional crystal structure for the Klenow fragment of *E. coli* polymerase I has been reported by Ollis *et al., Nature 313*:762-766 (1985) (reference AS7).[1] In this structure, regions that may be associated with polymerase and 3'-5' exonuclease activity are identified. Based on the sequence homology between *E. coli* polymerase I and the *Tma* polymerase, the *Tma* polymerase and *E. coli* polymerase I are expected to have a similar structure. Thus, this additional structural information provides extensive guidance to one skilled in the art to select mutants having reduced or eliminated 3'-5' exonuclease activity, while retaining polymerase activity. Based on the sequence homology between *E. coli* polymerase I and the *Tma* DNA polymerase, a scientist skilled in the art would readily be able to identify segments or specific residues of the *Tma* DNA polymerase involved in polymerase and 3'-5' or 5'-3' exonuclease activity.

Further guidance to select fragments or mutants of *Tma* DNA polymerase would be provided to a scientist skilled in the art from mutational analyses performed on *Tne* DNA polymerase and other Pol I-type DNA polymerases. Polesky *et al., J. Biol. Chem. 265*:14579-14591 (1990) (reference AR8) describe several mutations within the polymerase domain of the Klenow fragment of *E. coli* polymerase I. Mutations at residues 849, 668 and

[1] The Klenow fragment of *E. coli* polymerase I also contains the 3'-5' exonuclease domain. Thus, this article also provides useful information regarding the 3'-5' exonuclease active site.

882 show a large decrease in catalytic activity. Thus, several residues inyolved in polymerase activity have been identified. Each of these residues are within regions of high homology. Moreover, these residues are located near the region predicted to be associated with polymerase activity in the three-dimensional structure of the Klenow fragment. In a further article, several additional mutations are analyzed, confirming the location of the active site. *See* Polesky *et al., J. Biol. Chem. 267*:8417-8428 (1992) (reference AT7).

Mutational analyses have also been performed in the 3'-5' exonuclease domain of *E. coli* polymerase I. *See* Derbyshire *et al.*, *Science 240*:199-201 (1988) (reference AR3); and Derbyshire *et al.*, *EMBO J. 10*:17-24 (1990) (reference AT2). The mutations that resulted in loss of exonuclease activity, at residues 355, 424 and 501 were located in the regions of the 3'-5' exonuclease domain exhibiting a high degree of homology with *Tne* DNA polymerase and other members of the *E. coli* polymerase I family of polymerases. This information provides guidance to one skilled in the art to envision numerous specific mutations, in addition to those described in the specification, that will reduce or eliminate 3'-5' exonuclease activity.

Similarly, Pol I-type DNA polymerase mutants have been made containing mutations in the 5'-3' exonuclease domain. Several of these mutations, falling within regions of homology, reduce or eliminate 5'-3' exonuclease activity. Thus, these experiments also confirm that the selection of specific residues based on sequence homology can identify mutants expected to have reduced or eliminated 5'-3' exonuclease activity.

In light of this detailed and well known structural information for the Pol I-type polymerases, the structure/function correlations available for this family of polymerases, and the sequence and other structural information provided in this and the priority

applications for the *Tma* and *Tne* DNA polymerases, one skilled in the art can readily envision a representative number of members of the claimed genus.

Therefore, one skilled in the art would readily recognize that Applicants were in possession of the *Tma* DNA polymerase mutants specified in the claims and have satisfied the written description requirement of 35 U.S.C. § 112. *See Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 U.S.P.Q.2d 1111, 1118-19 (Fed. Cir. 1991); and *Union Oil Co. of California v. Atlantic Richfield Co.*, 54 U.S.P.Q.2d 1227 (Fed. Cir. 2000). Since the claimed invention is adequately described, withdrawal of the written description rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

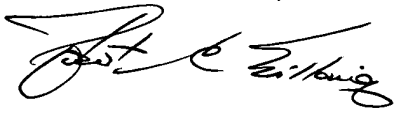
Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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